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(54) **PRESERVATIVE FOR LIVE CELL**

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a preservative, enabling the preservation of a live cell of fishes, etc., in especially a frozen state for a long period by using a specific component.
SOLUTION: This preservative comprises levan or a levan oligosaccharide (e.g. levan biose or levan octaose) as an active ingredient. The preservative is usually used as an aqueous solution and the concentration of the levan or levan oligosaccharide is 0.1-20wt.%. Thereby, the preservative has no toxicity to a cell and crystallinity is low. As a result, the live cell can efficiently be frozen and preserved and the preservative can readily be utilized without requiring washing even when culturing the cell after thawing thereof.

[JP,09-255501,A]

http://www4.ipdl.ncipi.go.jp/Tokujitu/PAJdetail.ipdl?N0000=60&N0120=01&N2001=2&N3001=H09-255501

CLAIMS

[Claim(s)]
[Claim 1] The preservative of the viable cell which contains levan or a levan oligosaccharide as an active principle.
[Claim 2] The preservative according to claim 1 whose viable cell is a fishes cell.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the preservative used in case it is low temperature and the viable cell of a viable cell, especially a fishes cell is especially saved in the state of freezing preferably at a long period of time. In addition, "freezing" in the cryopreservation agent indicated in this invention below is mind including "low temperature."
[0002]
[Description of the Prior Art] Although the viable cell has a living function and biological activity, it is various and there is utility value, on the other hand, under the conditions that temperature is high, it will change with time because of a metabolic turnover or denaturation, and a living function and biological activity will fall thru/or disappear. Then, although it is necessary to freeze and save a viable cell, DMSO (dimethyl sulfoxide), ethylene glycol, glycerol, etc. are used for the cryopreservation of a living thing cell as a cell membrane transparency mold cryopreservation agent from the former, and the polyvinyl pyrrolidone etc. is used as a cell nontransparent mold cryopreservation agent.
[0003]
[Problem(s) to be Solved by the Invention] However, since these cryopreservation agents are synthetic compounds, when it is generally made high concentration, they have the problem that toxicity is shown to a cell. Moreover, since it checks growth of a cell in cultivating, it is necessary to remove a cryopreservation agent from a cell beforehand, and becomes complicated. When the cryopreservation agent of a cell membrane transparency mold is furthermore used, there is a fault from which a cell tends to get a dilution shock by water in the case of defrosting since the elimination rate to the outside of a cell is slower than water.
[0004] Although, using the raffinose which is three saccharides as a natural product on the other hand is known (JP,5-38284,A), since this sugar is extracted from a beat etc., manufacture has constraint, and also since crystallinity is high, it has the problem that there is a possibility of hurting one's cell. Moreover, in this official report, although using 1-kestose as a viable cell preservative is proposed, since the price is still high, this also has the difficulty that it cannot use freely.
[0005] For this reason, even if there is no toxicity over a cell as a preservative of a viable cell or it was, what [crystalline / low] very low and was desirable, and when cultivating after

defrosting further, there is no need for washing, and what can be used freely was called for.
[0006]
[Means for Solving the Problem] this invention persons came to complete this invention, as a result of inquiring wholeheartedly, in order to solve said trouble.
[0007] That is, this invention relates to the preservative of the viable cell which contains levan or a levan oligosaccharide as an active principle. The levan or the levan oligosaccharide used in this invention fulfills said conditions, and it has the advantage that it can manufacture easily by the microorganism further. Especially as a levan oligosaccharide used in this invention, levan biose and levan OKUTAO-SU are desirable.
[0008] Levan is beta-2 of the fructofuranose and a kind of the polysaccharide which consists of a chain by 6-association which melt in water well, and was used [be / it / under / of the blood substitute / adding] conventionally. The structure expression of levan is as follows.
[0009]
[Formula 1]

[0010] Although there is especially no limit about the manufacture approach of of the levan or the levan oligosaccharide used for this invention, the approach (JP,3-219889,A) of producing using the microorganism belonging to the microorganism which belongs to the Serratia group for which these people applied, for example as the manufacture approach of levan and an Acetobacter group, an Aerobacter group, a Bacillus group, an Erwinia group, Zymomonas, etc. is mentioned. The approach (JP,7-115986,A) of producing using the microorganism belonging to the Streptomyces group for which these people similarly applied as the manufacture approach of levan OKUTAO-SU from levan can be mentioned. The approach (JP,5-244974,A, JP,8-279,A) of producing using the microorganism which belongs to the Streptomyces group for which these people applied as the manufacture approach of the levan biose from levan can be mentioned.
[0011] The viable cell preservative of this invention can usually be used as a water solution, and there is especially no limit about operating concentration. However, in order to demonstrate the effectiveness which was excellent as a viable cell preservative, it is usually desirable to use the concentration of levan or a levan oligosaccharide in 1 - 10wt% preferably 0.1 - 20wt%. If effectiveness will become weak if concentration is too low, and it becomes high concentration, it will be easy to become insoluble in water. In addition, levan or a levan oligosaccharide can also be used with other cryopreservation agents.
[0012]
[Embodiment of the Invention] Although an example is given to below and this invention is explained to it, this invention is not limited to these.
[0013] It dissolved in MEM10-Tris (commercial liquid medium) by 10% of concentration, respectively, using levan, levan OKUTAO-SU, levan biose, DMSO, and glycerol as an example 1 cryopreservation agent, and considered as the freezing medium. Germ origin cell CHSE-214 cell of a day [of culture / 5th] Chinock salmon is processed by the trypsin (EDTA is included), cell suspension is created, and it is Hank's once. After washing by BSS (commercial liquid medium), it suspended so that the concentration of a cell might become the liquid which added the aforementioned freezing medium to FBS (commercial blood serum) at 10% of a rate with 3.5x105-/ml. It poured distributively in the tube made from polypropylene, dedicated to the simple heat insulation box, and saved for one month at the deep freezer (-80 degrees C). an after that deep freezer to a tube -- taking out -- immediately -- a stream (about 15 degrees C) -- inside -- after fusion and 10ml Hank's BSS was added, and it washed once and re-suspended in the 5ml

culture medium (MEM10-Tris).
[0014] Measurement of a survival rate is Hank's. It is Hank's again in a part of cell washed by BSS. It suspended in BSS, one drop (50microl) of trypan blue liquid was added to 1ml of cell suspension 4%, the number of a **** cell (dead cell) and the cells (viable cell) not dyeing was measured after 1 minute using the blood cell count board, and the survival rate was measured from the average of eight visual fields. In addition, the 5ml of the above-mentioned cell suspension was scattered to 25cm3 **********, it dedicated to the after [standing] incubator (15 degrees C) at 15 degrees C for about 2 hours until the cell adhered to the container wall, and the subsequent growth condition was observed, and survivability was checked.
[0015] The experimental result of a survival rate was as follows. They were DMSO93% and glycerol 92% levan biose 83% levan OKUTAO-SU 85% levan 96%. The survival rate by levan is higher than DMSO and glycerol, and levan OKUTAO-SU and levan biose are also 80% or more of survival rates, and moreover, when there is no toxicity over a cell and crystallinity cultivates after defrosting further low, it has the advantage that there is no need for washing.
[0016] In example 2 example 1, the result of having experimented like the example 1 is shown below except replacing with Chinock salmon germ origin cell CHSE-214, and using the FHM cell of the **** origin of a fat head minnow. The experimental result of a survival rate was as follows. They were DMSO94% and glycerol 72% levan biose 50% levan OKUTAO-SU 58% levan 94%. The survival rate by levan is equipped with the description which it is the same as DMSO, and is quite higher than glycerol, and levan OKUTAO-SU and levan biose are also 50% or more, and was described in the example 1.
[0017] In example 3 example 1, the result of having experimented like the example 1 is shown below except replacing with Chinock salmon germ origin cell CHSE-214, and using RTG-2 cell of the ootid origin of a Rainbow trout. The experimental result of a survival rate is as follows, and is ******. They were DMSO90% and glycerol 74% levan biose 28% levan OKUTAO-SU 32% levan 91%. Like the example 2, the survival rate by levan is higher than DMSO and glycerol, and levan OKUTAO-SU and levan biose also have said advantage just over or below 30%.
[0018]
[Effect of the Invention] The preservative of this invention does not have the toxicity over a cell, and since crystallinity is low again, it can be saved in the state of freezing at the effectiveness which was excellent in the viable cell. Also when cultivating after thawing furthermore, washing is not needed, but the effectiveness that it can use freely is done so.

TECHNICAL FIELD

[Field of the Invention] This invention relates to the preservative used in case it is low temperature and the viable cell of a viable cell, especially a fishes cell is especially saved in the state of freezing preferably at a long period of time. In addition, "freezing" in the cryopreservation agent indicated in this invention below is mind including "low temperature."

PRIOR ART

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] However, since these cryopreservation agents are synthetic compounds, when it is generally made high concentration, they have the problem that toxicity is shown to a cell. Moreover, since it checks growth of a cell in cultivating, it is necessary to remove a cryopreservation agent from a cell beforehand, and becomes complicated. When the cryopreservation agent of a cell membrane transparency mold is furthermore used, there is a fault from which a cell tends to get a dilution shock by water in the case of defrosting since the elimination rate to the outside of a cell is slower than water.

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